

WHAT IS CLAIMED:

1 1. A recombinant ribonuclease that has (a) measurable ribonuclease
2 activity; (b) an amino terminal end beginning with a glutamine; (c) a leucine at position
3 11; an asparagine at position 21, a threonine at position 85, and a histidine at position 103,
4 such positions being determined through alignment with reference to those specified amino
5 acid positions of SEQ ID NO:2; and (d) substantial identity to SEQ ID NO:2.

1 2. The recombinant ribonuclease of claim 1, further comprising a
2 methionine residue at position 1.

1 3. The recombinant ribonuclease of claim 2, wherein the methionine
2 residue at position 23 as shown in SEQ ID NO:2 is replaced with a leucine residue.

1 4. The recombinant ribonuclease of claim 3, further comprising
2 histidine residues at 1 through 6 (SEQ ID NO:9).

1 5. The recombinant ribonuclease of claim 1, wherein the glutamine at
2 position 1 is cyclized to pyroglutamic acid.

1 6. The recombinant ribonuclease of claim 1, wherein the glutamine at
2 position 1 is replaced with a serine.

1 7. A cytotoxic reagent comprising the recombinant ribonuclease of
2 claim 1, linked to a ligand binding moiety.

1 8. The cytotoxic reagent of claim 7, further comprising a methionine
2 residue at position 1.

1 9. The cytotoxic reagent of claim 8, wherein the methionine residue at
2 position 23 as shown in SEQ ID NO:2 is replaced with a leucine residue.

09622613-073101

1 10. The cytotoxic reagent of claim 9, further comprising histidine
2 residues at 1 through 6.

1 11. The cytotoxic reagent of claim 7, wherein the glutamine at position 1
2 is cyclized to pyroglutamic acid.

1 12. The cytotoxic reagent of claim 7, wherein the glutamine at position 1
2 is replaced with a serine.

1 13. The cytotoxic reagent of claim 7, wherein the ribonuclease of SEQ
2 ID NO:2 is linked to a ligand binding moiety through a covalent bond.

1 14. The cytotoxic reagent of claim 13, wherein said covalent bond is at
2 the carboxy terminus of the ribonuclease of SEQ ID NO:2.

1 15. The cytotoxic reagent of claim 7, wherein said ligand binding moiety
2 is an antibody directed against a cell surface antigen present on a cancer cell.

1 16. The cytotoxic reagent of claim 15, wherein said antibody is a
2 recombinant single chain antibody.

1 17. The cytotoxic reagent of claim 15, wherein said antibody is directed
2 against a cell surface antigen on a cancerous B cell.

1 18. The cytotoxic reagent of claim 17, wherein said antibody is directed
2 against CD22.

1 19. The cytotoxic reagent of claim 18, wherein said antibody is LL2.

1 20. A nucleic acid which encodes a recombinant ribonuclease having a
2 nucleotide sequence as shown in SEQ ID NO:14 and conservative variants thereof.

09622613.073101

1 21. A recombinant ribonuclease encoded by a nucleic acid comprising
2 SEQ ID NO:14 and conservative variants thereof.

1 22. The ribonuclease of claim 21, wherein the amino acid sequence is
2 selected from the group consisting of SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19,
3 SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26.

1 23. A cytotoxic reagent comprising the ribonuclease of claim 22 linked
2 to a ligand binding moiety.

1 24. The cytotoxic reagent of claim 23, wherein the ribonuclease is linked
2 to a ligand binding moiety through a covalent bond.

1 25. The cytotoxic reagent of claim 24, wherein said covalent bond is at
2 the carboxy terminus of the ribonuclease.

1 26. The cytotoxic reagent of claim 23, wherein said ligand binding
2 moiety is an antibody directed against a cell surface antigen present on a cancer cell.

1 27. The cytotoxic reagent of claim 26, wherein said antibody is a
2 recombinant single chain antibody.

1 28. The cytotoxic reagent of claim 26, wherein said antibody is directed
2 against a cell surface antigen on a cancerous B cell.

1 29. The cytotoxic reagent of claim 28, wherein said antibody is directed
2 against CD22.

1 30. The cytotoxic reagent of claim 29, wherein said antibody is LL2.

1 31. A method of preparing a substantially pure recombinant
2 ribonuclease, said method comprising:

09522613.073101

- 3 (i) contacting said ribonuclease with an effective concentration
4 of a cleaving agent such that the ribonuclease is cleaved after the carboxy group of
5 methionine at position 1;
6 (ii) passing said ribonuclease through a Ni^{2+} -NTA agarose
7 column; and
8 (iii) eluting said substantially pure ribonuclease from said column.

1 32. A method of preparing a substantially pure recombinant cytotoxic
2 reagent, said method comprising:

- 3 (i) contacting said cytotoxic reagent with an effective
4 concentration of a cleaving agent such that the cytotoxic reagent is cleaved after the
5 carboxy group of methionine at position 1;
6 (ii) passing the cytotoxic reagent through a Ni^{2+} -NTA agarose
7 column; and
8 (iii) eluting said substantially pure cytotoxic reagent from said
9 column.

1 33. The method of claim 31, 32, wherein said cleaving agent is CNBr.

1 34. A pharmaceutical composition comprising a ribonuclease expressed
2 from recombinant DNA, said ribonuclease comprising a sequence selected from the group
3 consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID
4 NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID
5 NO:21, SEQ ID NO:24 and SEQ ID NO:26 in a pharmaceutically acceptable carrier.

1 35. The pharmaceutical composition of claim 34, further comprising an
2 antineoplast.

1 36. The pharmaceutical composition of claim 35, wherein said
2 antineoplast is Adriamycin.

09622613-073101

1 37. A pharmaceutical composition comprising a cytotoxic reagent, said
2 cytotoxic reagent comprising a sequence selected from the group consisting of SEQ ID
3 NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13,
4 SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:24 and
5 SEQ ID NO:26 in a pharmaceutically acceptable carrier.

1 38. A method of killing cancer cells comprising contacting cells to be
2 killed with a ribonuclease expressed by recombinant DNA and having a sequence selected
3 from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8,
4 SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ
5 ID NO:21, SEQ ID NO:24 and SEQ ID NO:26.

1 39. A method of killing cancer cells comprising contacting cells to be
2 killed with a cytotoxic reagent expressed by recombinant DNA, comprising a sequence
3 selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ
4 ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID
5 NO:19, SEQ ID NO:21, SEQ ID NO:24 and SEQ ID NO:26 covalently linked to a ligand
6 binding moiety, said ligand binding moiety directed against a cell surface antigen on the
7 cancer cells.

1 40. The method of claim 39, wherein said cancer cell is a malignant B
2 cell.

1 41. The method of claim 39, wherein said ligand binding moiety is an
2 antibody.

1 42. The method of claim 41, wherein said antibody is a single chain
2 antibody.

1 43. The method of claim 41, wherein said ligand binding moiety is an
2 antibody directed against CD22.

05622613-073101

1

44. The method of claim 43, wherein said antibody is LL2.

09622613.073101